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(54) MANGANESE PEROXIDASE, GENE THEREOF, AND USE THEREOF IN DETOXIFICATION OF MYCOTOXIN

(57) The present invention provides use of a manganese peroxidase in the detoxification of mycotoxins, and specifically, the present invention provides five manganese peroxidases (MnP-1, MnP-2, MnP-4, MnP-5, and MnP-6), genes thereof, and uses thereof. The present invention provides five manganese peroxidases (MnP-1, MnP-2, MnP-4, MnP-5, and MnP-6) derived from lignocellulose degradation bacteria, the amino acid sequences thereof being as set forth in SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 10, and SEQ ID NO: 13.

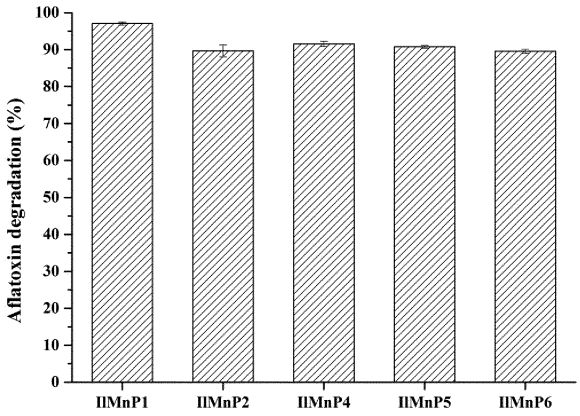

FIG 1

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to the field of genetic engineering, particularly to five manganese peroxidases, i.e. MnP-1 MnP-2 MnP-4 MnP-5 and MnP-6, genes thereof, vector containing these genes, and application thereof.

BACKGROUND OF THE INVENTION

[0002] Mycotoxin is a kind of fugal secondary metabolite with the different structure and properties, which is harmful to the health of livestock, poultry and human, and is widely spread in the food and the feed contaminated by mold, thus attracting the worldwide concern on the safety of the food and the feed. The common mycotoxins include aflatoxin, zearalenone, vomitoxin (deoxynivalenol), citrinin, ochratoxin, fumaricin, patulin, and monosporotoxin, which may be classified into two types of toxins with or without a ring structure. Most of mycotoxins, such as aflatoxin and zearalenone, belong to the sub-group with the ring structure, and are usually synthesized by the fungal polyketo pathway. Aflatoxin B1, for example, is a strong liver carcinogen produced by Aspergillus flavus, with a core coumarin ring, two five-carbon rings on its both sides and two side-by-side dihydrofuran rings. In addition, zearalenone is a mesodihydroxybenzoate phenolide. In contrast, fumonisin has P- aminophenol linear skeleton of 22 carbons with two malonic acids side chains.

[0003] Physical adsorption (or inactivation) and bioconversion are the two main ways to detoxify mycotoxins in the food and feed. It is an increasingly popular method of detoxifying mycotoxins by using microorganisms, especially enzymes produced by microorganisms. It has been reported that laccase, pan-lylytic lactone hydrolase, peroxidase and some enzymes not yet classified can degrade aflatoxin and zearalenone by oxidation or hydrolysis mechanism. There are more and more evidences demonstrating that other enzymes with unknown properties may also be involved in the degradation of aflatoxin and zearalenone, which are two kinds of mycotoxins with cyclic structure. Manganese peroxidase (MnP) from lignocellulose-degrading bacteria is a group of enzymes involved in the oxidative degradation of lignin. It has been found that a few manganese peroxidases from Phanerochaete sordida and Pleurotus ostreatus are capable to degrade aflatoxin, but it is still not known whether other manganese peroxidases capable of degrading mycotoxins.

[0004] Irpex lacteus is a kind of white rot fungus, which can effectively degrade lignocellulose. Biochemical, genomic and transcriptomics analyses shows that manganese peroxidase may play an important role in the degradation of lignin. Manganese peroxidase can be used not only in degradation of lignin but also in the remediation of environmental pollution caused by synthetic dyes and polycyclic aromatic hydrocarbons. The present invention cloned and expressed five manganese peroxidase genes from Irpex lacteus, and analyzed their ability to degrade aflatoxin, zearalenone, and vomitoxin.

Order of the invention

[0005] One order of the present invention is to provide five manganese peroxidases that can be efficiently applied to the detoxification of mycotoxin.

[0006] Another order of the present invention is to provide genes encoding the above manganese peroxidases.

[0007] Another order of the present invention is to provide a recombinant vector comprising the above genes.

[0008] Another order of the present invention is to provide a recombinant strain comprising the above gene.

[0009] Another order of the present invention is to provide a method of preparing the above manganese peroxidases.

[0010] Another order of the present invention is to provide application of the above manganese peroxidases to detoxify mycotoxin.

SUMMARY OF THE INVENTION

[0011] The present invention isolated five novel manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6, with the amino acids sequence as shown in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.10 and SEQ ID NO.13, respectively.

SEQ ID NO.1(MnP-1):

MAFKTILAFVALATAALAAPSSRVTCSPGRVVSNGACCKWFDVLDDIQENLFD
GGVCGEEVHESLRLTFHDAIGFSLSAEREGKFGGGGADGSIMAFAEIETNFHA
NNGVDEIVEAQRPFAIKHKVSFGDFIQFAGAVGVSNCLGGPRLEFMAGRSNIS
RAAPDLTVPEPSDSVDKILARMGDAGFSSSEVVDLLISHTVAAQDHVDPTIPGT
PFDSTPSEFDPQFFVETLLKGTLFPGNGSNVGELQSPLRGEFRLQSDALLARDP

RTACEWQSFVNNQRLMVTKFEAVMSKLAVLGHNPRDLVDCSEVIPVPPRAKT
NVAVLPAGKTRADVQAACAATPFPTLQTAPGPATSIVPV

[0012] Manganese peroxidases MnP-1 includes 358 amino acids with a signal peptide of 18 amino acids, "MAFKTILAFVALATAALA", in N-terminal, as set in forth in SEQ ID NO.2.

SEQ ID NO. 2
MAFKTILAFVALATAALA

[0013] Thereof, the mature manganese peroxidases MnP-1 protein has the amino acids as shown in SEQ ID NO.3, and a theoretical molecular weight of 36.1kDa.

SEQ ID NO.3:

APSSRVTCSPGRVVSNGACCKWFDVLDDIQENLFDGGVCGEEVH
ESLRLTFHDAIGFSLSAEREGKFGGGGADGSIMAFAEIETNFHANN
GVDEIVEAQRPFAIKHKVSFGDFIQFAGAVGVSNCLGGPRLEFMA
GRSNISRAAPDLTVPEPSDSVDKILARMGDAGFSSSEVVDLLISHT
VAAQDHVDPTIPGTPFDSTPSEFDPQFFVETLLKGTLFPGNGSNVG
ELQSPLRGEFRLQSDALLARDPRTACEWQSFVNNQRLMVTKFEA
VMSKLAVLGHNPRDLVDCSEVIPVPPRAKTNVAVLPAGKTRADV
QAACAATPFPTLQTAPGPATSIVPV

SEQ ID NO.4(MnP-2):

MAFKHLVVALSIVLSLGVAQAAITKRVACPDGKNTATNAACCSL
FAIRDDIQANLFDGGECGEEVHESFRLTFHDAIGTGSFGGGGADG
SIIVFDDIETNFHANNGVDEIIDEQKPFIARHNITPGDFIQFAGAVGV
SNCPGAPRLDFFLGRPNPVAAAPDKTVPEPFDTVDSILARFKDAG
GFTPAEIVALLGSHTIAAADHVDPTIPGTPFDSTPEVFDTQVFVEV
QLRGTLFPGTGGNQGEVQSPLRGEIRLQSDHDLARDSRTACEWQS

FVNNQAKLQSAFKAAFKKLSVLGHNINNLIDCSEVIPEPPNVKVK
PATFPAGITHADVEQACATTPFPTLATDPGPATSVAPVPPS

[0014] Manganese peroxidase MnP-2 contains 359 amino acids with a signal peptide of 21amino acids, "MAFKHLV-VALSIVLSLGVAQA", in N-terminal, as set in forth in SEQ ID NO.5.

[0015] Thereof, the mature manganese peroxidase MnP-2 protein has the amino acids as shown in SEQ ID NO.6, and a theoretical molecular weight of 35.6kDa.

SEQ ID NO.6：

AITKRVACPDGKNTATNAACCSLFAIRDDIQANLFDGGECGEEVH
ESFRLTFHDAIGTGSFGGGGADGSIIVFDDIETNFHANNGVDEIIDE
QKPFIARHNITPGDFIQFAGAVGVSNCPGAPRLDFFLGRPNPVAAA
PDKTVPEPFDTVDSILARFKDAGGFTPAEIVALLGSHTIAAADHVD
PTIPGTPFDSTPEVFDTQVFVEVQLRGTLFPGTGGNQGEVQSPLRG
EIRLQSDHDLARDSRTACEWQSFVNNQAKLQSAFKAAFKKLSVL
GHNINNLIDCSEVIPEPPNVKVKPATFPAGITHADVEQACATTPFPT
LATDPGPATSVAPVPPS

SEQ ID NO.7(MnP-4)：

MTFKALLALLTVTSAVLAAPQDVTAANKVSCGGGRVAGHAQCC
KWYDVLDDIQKNLFDGGECGEEVHESLRLTFHDAIGFSLSAQREG
KFGGGGADGSIMAFAEIETKFHANNGVDEIIEAQRPFALNHSVSFG
DFIQFAGAVGVSNCGGGPRLQFLAGRSNSSKAAPDGTVPEPFDST
DKILAHMGDAGFSPSEVVDLLASHSVAAQDHVDASIPGTPFDSTP
STFDAQFFVETLLKGTLFPGNGSNQGEVQSPLHGEFRLQSDFELA
RDSRTACEWQSFITDHNSMVRKFEAAMAKLAVLGHDPRTLIDCS
DVIPQPKGAKSNVAVLPAGKHRADIQASCHQTPFPTLKTAPGPET
SIPPVPPS

[0016] Manganese peroxidase MnP-4 contains 365 amino acids with a signal peptide of 18 amino acids, "MTFKA-LLALLTVTSAVLA", in N-terminal, as set in forth in SEQ ID NO.8

[0017] Thereof, the mature manganese peroxidase MnP-4 protein has the amino acids as shown in SEQ ID NO.9, and a theoretical molecular weight of 36.8kDa.

SEQ ID NO.9：

APQDVTAANKVSCGGGRVAGHAQCCKWYDVLDDIQKNLFDGG
ECGEEVHESLRLTFHDAIGFSLSAQREGKFGGGGADGSIMAFAEIE
TKFHANNGVDEIIEAQRPFALNHSVSFGDFIQFAGAVGVSNCGGG
PRLQFLAGRSNSSKAAPDGTVPEPFDSTDKILAHMGDAGFSPSEV
VDLLASHSVAAQDHVDASIPGTPFDSTPSTFDAQFFVETLLKGTLF
PGNGSNQGEVQSPLHGEFRLQSDFELARDSRTACEWQSFITDHNS
MVRKFEAAMAKLAVLGHDPRTLIDCSDVIPQPKGAKSNVAVLPA
GKHRADIQASCHQTPFPTLKTAPGPETSIPPVPPS

SEQ ID NO.10(MnP-5)：

MAFKQLVATLSLALLAHGAVVRRVTCPDGVNTATNAACCSLFA
VRDDIQQNLFDNGQCGEDVHESFRLSFHDAIGISPKIAATGQFGGG
GADGSIILFEEIETNFHANIGVDEIVDEQKPFIARHNITPGDFIQFAA
AVGVSNCPGAPRLDFFLGRPAATQPAPDKTVPEPFDTVDTILERFA
DAGNFTPAEVVALLVSHTIAAADEVDPTIPGTPFDSTPEVFDSQFF
VETQLRGTGFPGTAGNQGEVESPLAGELRLQSDSELARDSRTACE
WQSFVGNQQKIQTAFKAAFQKMAVLGVDTSKMVDCSELIPVPPE
LKITAAHFPAGKTNADVEQACASTPFPTLSTDPGPATSVAPVPPS

[0018] Manganese peroxidase MnP-5 contains 363 amino acids with a signal peptide of 18 amino acids, "MAFKQL-VATLSLALLAHG", in N-terminal, as set in forth in SEQ ID NO.11.

[0019] Thereof, the mature manganese peroxidase MnP-5 protein has the amino acids as shown in SEQ ID NO.12, and a theoretical molecular weight of 36.5kDa.

SEQ ID NO.12：

AVVRRVTCPDGVNTATNAACCSLFAVRDDIQQNLFDNGQCGED
VHESFRLSFHDAIGISPKIAATGQFGGGGADGSIILFEEIETNFHANI
GVDEIVDEQKPFIARHNITPGDFIQFAAAVGVSNCPGAPRLDFFLG
RPAATQPAPDKTVPEPFDTVDTILERFADAGNFTPAEVVALLVSH
TIAAADEVDPTIPGTPFDSTPEVFDSQFFVETQLRGTGFPGTAGNQ
GEVESPLAGELRLQSDSELARDSRTACEWQSFVGNQQKIQTAFKA
AFQKMAVLGVDTSKMVDCSELIPVPPELKITAAHFPAGKTNADV
EQACASTPFPTLSTDPGPATSVAPVPPS

SEQ ID NO.13(MnP-6)：

MAFKQLVAALTVALSLGVAQGAITRRVACPDGVNTATNAACCSL
FAIRDDIQQNLFDGGECGEEVHESFRLTFHDAIGIGSNGGGGADGS
IAVFEDIETAFHANNGVDEIIDEQKPFLARHNITPGDFIQFAGAVGV
SNCPGAPRLDFFLGRPNPVAPAPDKTVPEPFDTVDSILARFADAGG
FSPAEVVALLGSHTIAAADHVDPTIPGTPFDSTPEVFDTQVFLEVQ
LRGTLFPGTGGNQGEVESPLRGEIRLQSDHDLARDSRTACEWQSF
VNNQVKLQTAFKAAFKKLAVLGHDVNNMVDCSEVIPEPPNVKIK
AATFPAGQTNADVEQACASTPFPTLATDPGPATSVAPVPPS

[0020] Manganese peroxidase MnP-6 contains 359 amino acids with a signal peptide of 21 amino acids, "MAFKQLVAALTVALSLGVAQG", in N-terminal, as set in forth in SEQ ID NO.14.

[0021] Thereof, the mature manganese peroxidase MnP-6 protein has the amino acids as shown in SEQ ID NO.15, and a theoretical molecular weight of 36.5kDa

SEQ ID NO.15：

AITRRVACPDGVNTATNAACCSLFAIRDDIQQNLFDGGECGEEVHE
SFRLTFHDAIGIGSNGGGGADGSIAVFEDIETAFHANNGVDEIIDEQ
KPFLARHNITPGDFIQFAGAVGVSNCPGAPRLDFFLGRPNPVAPAP
DKTVPEPFDTVDSILARFADAGGFSPAEVVALLGSHTIAAADHVDP
TIPGTPFDSTPEVFDTQVFLEVQLRGTLFPGTGGNQGEVESPLRGEI
RLQSDHDLARDSRTACEWQSFVNNQVKLQTAFKAAFKKLAVLGH
DVNNMVDCSEVIPEPPNVKIKAATFPAGQTNADVEQACASTPFPT
LATDPGPATSVAPVPPS

[0022] Yet another aspect of the invention is to provide the genes encoding the above manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6. Particularly, the gene encoding manganese peroxidase MnP-1 has a nucleotide sequence set in forth in SEQ ID NO.16

SEQ ID NO.16(MnP-1)：

atggctttcaagactatccttgccttcgttgctctcgccacagctgctcttgcggcaccctcttctagagtgacatgcagtccg
ggacgtgttgttagcaacggagctgtaagcaattctcgacaccgtcctaccaattataacgtctaatggccgtcgtactagt
gctgcaagtggttcgacgttctcgacgacatccaggagaacctgtatgtccttcccgttgctcagtgaaccttgtcgccgctg
attccatcacaggtttgacggcggtgtatgtggcgaagaagttcacgaggtaagtaacgattacagcaggtagttgatgca
tactaacagttgctctttgcagtcgcttcgtgtaagtgactctcagaatgaacgtggtgaacgcatattgacatgtgccttcc
attgccaagctcactttccacgacgcgtaagtgtctgttgtcactattcttgcttcttgtgctgatcctgtctgtatagtattggc
tttagtctctctgctgagcgcgagggcaagtttgggttcgtacttcaacttcacaatgtccctttttgatgattcacatccgcct
atagtggtggaggagctgatggctctatcatggcattcgccgagattgagaccaacttccgtgcgtaaacctgggcctttgt
tgagtgcttatattaaactctgaagcagatgcaaacaatggtgtcgacgaaattgtcgaggcggtatgtctcttcatgtgtc
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tggtaatcaggcatattattgagcaatactcatcacgacatctacagggaacacctttgactccacccccctccgaattcga
tcctcagttcttcgtagaggtaagctttgaccacgtcatcgtcaagcgaagcgacttaagggtctttttacagactctcttga
agggcactctgttccctggtaacggttccaatgtcggcgaacttcagtccccccttagaggagagttccgtcttcaatccga

cgctctccttgctcgtgaccccaggaccgcctgtgaatggcaatctttcgttagtgagtatcctcttcactttcatgtcgagac
tctataattgatgcacccgcctgtcagacaaccaacgtctcatggtcaccaagttcgaggccgtcatgtccaagcttgctgt
cctcggccacaacccgcgtgatctcgtcgactgctcggaagtcatccccgtgcctccacgtgccaagaccaatgtcgcagtt
ctccccgctggcaagactcgcgctgatgtccaggctgcttgcgctgctacacccttcccaaccctccagaccgcccctggcc
ccgccacctccatcgttcctgtgtaa

[0023] According to an embodiment, gene encoding manganese peroxidase MnP-1 was cloned by PCR, and the analysis of its DNA sequence showed its genomic sequence had full length of 1684bp, consisting of coding sequence of 1077bp, and an oligonucleotide sequence encoding the signal peptide as below.

SEQ ID NO.17：

ATGGCTTTCAAGACTATCCTTGCCTTCGTTGCTCTCGCCACAGC

TGCTCTTGCG

[0024] The cDNA sequence of the mature manganese peroxidase MnP-1 has a nucleotide sequence set in forth in SEQ ID NO.18

SEQ ID NO.18：

gcaccctcttctagagtgacatgcagtccgggacgtgttgttagcaacggagcttgctgcaagtggttcgacgttctcgacg
acatccaggagaacctgtttgacggcggtgtatgtggcgaagaagttcacgagtcgcttcgtctcactttccacgacgctat
tggctttagtctctctgctgagcgcgagggcaagtttggtggtggaggagctgatggctctatcatggcattcgccgagatt
gagaccaacttccatgcaaacaatggtgtcgacgaaattgtcgaggcgcaacgcccattcgctatcaagcacaaagtctc
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gatgctggcttttcctcttcggaagttgtggaccttctcatttcccacaccgttgcagctcaagaccacgttgatcccaccatc
cccggaacacctttgactccacccccctccgaattcgatcctcagttcttcgtagagactctcttgaagggcactctgttccct
ggtaacggttccaatgtcggcgaacttcagtccccccttagaggagagttccgtcttcaatccgacgctctccttgctcgtga
ccccaggaccgcctgtgaatggcaatctttcgttaacaaccaacgtctcatggtcaccaagttcgaggccgtcatgtccaa
gcttgctgtcctcggccacaacccgcgtgatctcgtcgactgctcggaagtcatccccgtgcctccacgtgccaagaccaat

gtcgcagttctccccgctggcaagactcgcgctgatgtccaggctgcttgcgctgctacacccttcccaaccctccagaccg
cccctggccccgccacctccatcgttcctgtgtaa

[0025] The mature manganese peroxidase MnP-1 protein has a theoretical molecular weight of 36.1kDa and is a novel manganese peroxidase.

SEQ ID NO.19(MnP-2):

atggccttcaaacacctcgtcgttgcactctctatcgttctctcgcttggtgtcgcacaaggtcagtagctcatggaataatg
cgcctgctaacttcgctgatgggactatgttgcagctgcaatcaccaagcgtgttgcttgtcctgacggcaagaatacagcg
acaaacgcggcttgctgttctttgttcgccattcgtgatgatatccaggcaaacctcttcgacggtggtgaatgcggtgaag
aagtccacgagtccttccgtctgtcagtacttggactcttctaacgtatcacttgtgaaattcatgcatgttttcagcacattcc
acgacgctatcggtactggctctttcgggtgagagatcaaagcttttatattgtgtactctacgcctgacatttgattatagtg
gcggaggtgccgatggctccatcattgtcttcgatgatatcgagactaacttccacgctaacaacggcgtcgacgaaattat
cgacgagcagaagccgttcatcgccaggcacaatattacccccggcgacttgtgagctgatcttgctattctatcgcattct
gaccactaatatatacactgatttcagcattcaatttgctggcgccgtcggcgtctccaactgtcctggggctcctcgtcttg
acttcttcctcggtaagactcatttcaataccgacaatgggcccatactgatgatacgatatccaggccgaccaaaccctgt
ggctgctgcaccggacaagactgtacctgagccattcggtcagtacaccaatcttcatcgtatctactccaaagctgatgta
agggcccctagacaccgtggatagcatccttgctcgtttcaaggatgctggcggattcactccagctgaggtagttgctctc
ctcggctctcacacgatcgctgcagccgatcatgtcgaccctaccatccctggtactcctttcgattctactcctgaggtcttc
gatacccaggttttcgtcgaggttcaactccgtggcacgctcttcccagggtgagtttcctgttttataacacatacctgagtc
tgactgcgacttgcccattagaactggtggcaaccagggcgaagttcagtctcctctccgcggtgagatccgtctccaatct
gaccatgatgtacgtgtacgatggatatttcgttctgggtcttactgacaagccttaagctcgctcgtgactctcgaaccgcc
tgcgagtggcagtcgtttgtgaacaaccaggctaagctccaatctgctttcaaagcagccttcaagaagctctcagtccttg
gccacaacattaacaacttgattgactgctctgaggtcatccctgagccaccaaatgtcaaggttaagcccgctaccttccc
agctggcattacccacgccgatgtcgagcaagctgtacgtgctctttctcctttgcttcctctatactcctaataatctgtttca
ctttgtagtgcgccactactccattcccgactctcgctaccgaccccggccccgcaacttctgtcgcccctgtgtaagttaca
tctttgacttcatgttacattatatgctcatatcgctttcagccctccctcgtaa

[0026] According to an embodiment, gene encoding manganese peroxidase MnP-2 was cloned by PCR, and the analysis of its DNA sequence showed genomic sequence had full length of 11692bp, consisting of the coding sequence of 1080bp, and an oligonucleotide sequence as below, encoding the signal peptide.

SEQ ID NO.20

ATGGCCTTCAAACACCTCGTCGTTGCACTCTCTATCGTTCTCTCGCTTGGTGTCGCACAAGCT

[0027] The cDNA sequence of the mature manganese peroxidase MnP-1 has a nucleotide sequence set in forth in SEQ ID NO.21.

SEQ ID NO.21:

Gcaatcaccaagcgtgttgcttgtcctgacggcaagaatacagcgacaaacgcggcttgctgttctttgttcgccattcgtg
atgatatccaggcaaacctcttcgacggtggtgaatgcggtgaagaagtccacgagtccttccgtctcacattccacgacg
ctatcggtactggctctttcggtggcggaggtgccgatggctccatcattgtcttcgatgatatcgagactaacttccacgct
aacaacggcgtcgacgaaattatcgacgagcagaagccgttcatcgccaggcacaatattacccccggcgacttcattca
atttgctggcgccgtcggcgtctccaactgtcctggggctcctcgtcttgacttcttcctcggccgaccaaaccctgtggctg
ctgcaccggacaagactgtacctgagccattcgacaccgtggatagcatccttgctcgtttcaaggatgctggcggattcac
tccagctgagatagttgctctcctcggctctcacacgatcgctgcagccgatcatgtcgaccctaccatccctggtactccttt
cgattctactcctgaggtcttcgatacccaggttttcgtcgaggttcaactccgtggcacgctcttcccaggaactggtggca
accagggcgaagttcagtctcctctccgcggtgagatccgtctccaatctgaccatgatctcgctcgtgactctcgaaccgc
ctgcgagtggcagtcgtttgtgaacaaccaggctaagctccaatctgctttcaaagcagccttcaagaagctctcagtcctt
ggccacaacattaacaacttgattgactgctctgaggtcatccctgagccaccaaatgtcaaggttaagcccgctaccttcc
cagctggcattacccacgccgatgtcgagcaagcttgcgccactactccattcccgactctcgctaccgaccccggccccg
caacttctgtcgcccctgtccctccctcgtaa

[0028] The mature manganese peroxidase MnP-2 protein has a theoretical molecular weight of 35.6kDa and is a novel

manganese peroxidase.

SEQ ID NO.22(MnP-4):

ATGACTTTCAAGGCTCTTCTTGCTCTTTTGACGGTTACTTCTGCGGTGCTCGCCGCTCCCCAAG
ACGTTACTGCCGCTAACAAGGTATCATGCGGTGGAGGCCGTGTCGCAGGTCATGCTCAATGCT
GCAAGTGGTATGACGTTCTCGACGACATACAGAAGAATTTGTTTGACGGTGGAGAATGCGGT
GAAGAAGTTCACGAGTCTTTGCGACTGACTTTCCACGACGCGATCGGCTTCAGTCTTTCGGCC
CAGCGTGAAGGGAAATTCGGCGGTGGAGGAGCTGACGGCTCTATCATGGCCTTCGCAGAGA
TCGAGACTAAATTTCACGCTAACAACGGTGTCGACGAGATCATTGAAGCTCAACGCCCCTTCG
CCCTCAACCACAGCGTGTCCTTCGGAGATTTCATCCAGTTCGCTGGTGCAGTCGGTGTTTCCA
ACTGTGGCGGCGGCCCTCGACTGCAGTTCTTGGCCGGTCGATCTAACAGCTCCAAGGCCGCA
CCTGATGGCACTGTCCCTGAGCCATTTGACTCTACTGATAAGATCCTCGCTCACATGGGCGACG
CTGGTTTCTCTCCGAGTGAAGTGGTCGATCTCTTGGCATCTCATTCCGTGGCTGCACAGGACC
ATGTCGACGCTTCTATCCCGGGAACCCCATTCGATTCTACTCCCAGCACATTCGATGCCCAATTC
TTTGTGGAGACTTTGCTGAAGGGCACGCTTTTCCCTGGAAATGGCTCTAACCAAGGCGAAGT
CCAGTCCCCTCTTCACGGAGAATTCCGCCTTCAGTCCGACTTTGAGCTCGCTCGTGACTCCCG
CACTGCTTGCGAGTGGCAGTCCTTCATCACCGATCACAACTCGATGGTTCGCAAGTTCGAAGC
CGCTATGGCCAAGCTAGCTGTTCTCGGTCACGACCCCCGCACTTTGATTGACTGTTCCGATGTC
ATTCCTCAACCCAAGGGTGCCAAATCTAACGTGGCTGTACTTCCGGCTGGAAAGCACCGTGC
GGATATTCAAGCATCTTGCCATCAAACGCCGTTTCCCACCCTCAAGACCGCTCCCGGACCCGA
GACCTCGATTCCTCCAGTACCTCCGTCGTAA

According to an embodiment, gene encoding manganese peroxidase MnP-4 was cloned by PCR, and the analysis of its DNA sequence showed genomic sequence had full length of 1760bp, consisting of the coding sequence of 1101bp, and an oligonucleotide sequence as below, encoding the signal peptide.

SEQ ID NO.23

ATGACTTTCAAGGCTCTTCTTGCTCTTTTGACGGTTACTTCTGCGGTGCTCGCC

[0029] The cDNA sequence of the mature manganese peroxidase MnP-4 has a nucleotide sequence set in forth in SEQ ID NO.24.

SEQ ID NO.24：

GCTCCCCAAGACGTTACTGCCGCTAACAAGGTATCATGCGGTGGAGGCCGTGTCGCAGGTCA
TGCTCAATGCTGCAAGTGGTATGACGTTCTCGACGACATACAGAAGAATTTGTTTGACGGTGG
AGAATGCGGTGAAGAAGTTCACGAGTCTTTGCGACTGACTTTCCACGACGCGATCGGCTTCA
GTCTTTCGGCCCAGCGTGAAGGGAAATTCGGCGGTGGAGGAGCTGACGGCTCTATCATGGC
CTTCGCAGAGATCGAGACTAAATTTCACGCTAACAACGGTGTCGACGAGATCATTGAAGCTCA
ACGCCCCTTCGCCCTCAACCACAGCGTGTCCTTCGGAGATTTCATCCAGTTCGCTGGTGCAGT
CGGTGTTTCCAACTGTGGCGGCGGCCCTCGACTGCAGTTCTTGGCCGGTCGATCTAACAGCT
CCAAGGCCGCACCTGATGGCACTGTCCCTGAGCCATTTGACTCTACTGATAAGATCCTCGCTCA
CATGGGCGACGCTGGTTTCTCTCCGAGTGAAGTGGTCGATCTCTTGGCATCTCATTCCGTGGC
TGCACAGGACCATGTCGACGCTTCTATCCCGGGAACCCCATTCGATTCTACTCCCAGCACATTC
GATGCCCAATTCTTTGTGGAGACTTTGCTGAAGGGCACGCTTTTCCCTGGAAATGGCTCTAAC
CAAGGCGAAGTCCAGTCCCCTCTTCACGGAGAATTCCGCCTTCAGTCCGACTTTGAGCTCGCT
CGTGACTCCCGCACTGCTTGCGAGTGGCAGTCCTTCATCACCGATCACAACTCGATGGTTCGC
AAGTTCGAAGCCGCTATGGCCAAGCTAGCTGTTCTCGGTCACGACCCCCGCACTTTGATTGAC
TGTTCCGATGTCATTCCTCAACCCAAGGGTGCCAAATCTAACGTGGCTGTACTTCCGGCTGGA
AAGCACCGTGCGGATATTCAAGCATCTTGCCATCAAACGCCGTTTCCCACCCTCAAGACCGCT
CCCGGACCCGAGACCTCGATTCCTCCAGTACCTCCGTCGTAA

[0030] The mature manganese peroxidase MnP-4 protein has a theoretical molecular weight of 36.8kDa and is a novel manganese peroxidase.

SEQ ID NO.25(MnP-5)：

ATGGCCTTCAAACAACTCGTTGCTACGCTCTCTCTCGCTCTCCTCGCCCATG
GTGCCGTCGTCAGGCGTGTCACTTGTCCCGACGGAGTGAACACAGCCACC
AACGCAGCTTGCTGCTCTTTGTTCGCCGTTCGTGACGATATCCAGCAGAAC
CTCTTCGACAACGGCCAATGCGGTGAAGACGTCCACGAATCTTTCCGTCTC
TCCTTCCACGATGCCATCGGAATCTCTCCCAAGATTGCGGCAACCGGCCAG
TTTGGAGGTGGAGGCGCTGACGGCTCTATCATCCTCTTTGAGGAGATTGAG
ACCAACTTCCACGCTAACATTGGTGTTGACGAGATTGTCGACGAGCAGAA
GCCGTTCATCGCCAGGCACAACATCACCCCCGGAGACTTCATCCAATTTGC
CGCCGCTGTTGGTGTCTCGAACTGCCCTGGTGCTCCTCGTCTCGACTTCTT
CCTTGGCCGTCCCGCTGCTACTCAACCCGCTCCAGACAAGACTGTCCCCGA
GCCCTTCGACACCGTCGACACCATCCTGGAACGTTTTGCAGATGCGGGAAA
TTTCACCCCAGCCGAGGTCGTCGCTCTCCTCGTCTCCCATACCATCGCTGCT
GCCGATGAGGTGGATCCCACCATCCCGGGAACTCCCTTCGACTCTACCCCG
GAGGTCTTCGACTCGCAGTTCTTCGTCGAGACTCAGCTTCGCGGAACAGG
ATTCCCAGGAACCGCGGGTAACCAAGGTGAAGTCGAATCTCCTCTTGCTGG
AGAACTGCGTCTCCAGTCCGACTCAGAGCTCGCTCGTGACTCCAGAACCG
CCTGCGAGTGGCAATCCTTCGTCGGCAACCAGCAGAAGATCCAAACCGCG
TTCAAGGCCGCTTTCCAGAAGATGGCCGTTCTCGGGGTAGACACCAGCAA
GATGGTCGACTGCTCCGAGCTCATTCCTGTTCCTCCTGAGCTGAAGATCAC
CGCCGCGCATTTCCCTGCTGGCAAGACCAACGCTGACGTCGAGCAAGCTT
GTGCTTCGACCCCCTTCCCCACTCTGTCCACTGACCCCGGCCCGGCTACTT
CTGTCGCTCCTGTCCCTCCGTCCTAA

[0031] According to an embodiment, gene encoding manganese peroxidase MnP-5 was cloned by PCR, and the analysis of its DNA sequence showed genomic sequence had full length of 1862bp, including encoding sequence of1092bp, and an oligonucleotide sequence as below, encoding the signal peptide.

SEQ ID NO.26

ATGGCCTTCAAACAACTCGTTGCTACGCTCTCTCTCGCTCTCCTCGCCCATG
GT

[0032] The cDNA sequence of the mature manganese peroxidase MnP-5 has a nucleotide sequence set in forth in SEQ ID NO.27.

SEQ ID NO.27：

GCCGTCGTCAGGCGTGTCACTTGTCCCGACGGAGTGAACACAGCCACCAACGCAGCTT
GCTGCTCTTTGTTCGCCGTTCGTGACGATATCCAGCAGAACCTCTTCGACAACGGCCAATGCG
GTGAAGACGTCCACGAATCTTTCCGTCTCTCCTTCCACGATGCCATCGGAATCTCTCCCAAGAT
TGCGGCAACCGGCCAGTTTGGAGGTGGAGGCGCTGACGGCTCTATCATCCTCTTTGAGGAGA
TTGAGACCAACTTCCACGCTAACATTGGTGTTGACGAGATTGTCGACGAGCAGAAGCCGTTC
ATCGCCAGGCACAACATCACCCCCGGAGACTTCATCCAATTTGCCGCCGCTGTTGGTGTCTCG
AACTGCCCTGGTGCTCCTCGTCTCGACTTCTTCCTTGGCCGTCCCGCTGCTACTCAACCCGCTC
CAGACAAGACTGTCCCCGAGCCCTTCGACACCGTCGACACCATCCTGGAACGTTTTGCAGAT
GCGGGAAATTTCACCCCAGCCGAGGTCGTCGCTCTCCTCGTCTCCCATACCATCGCTGCTGCC
GATGAGGTGGATCCCACCATCCCGGGAACTCCCTTCGACTCTACCCCGGAGGTCTTCGACTCG
CAGTTCTTCGTCGAGACTCAGCTTCGCGGAACAGGATTCCCAGGAACCGCGGGTAACCAAG
GTGAAGTCGAATCTCCTCTTGCTGGAGAACTGCGTCTCCAGTCCGACTCAGAGCTCGCTCGTG
ACTCCAGAACCGCCTGCGAGTGGCAATCCTTCGTCGGCAACCAGCAGAAGATCCAAACCGCG
TTCAAGGCCGCTTTCCAGAAGATGGCCGTTCTCGGGGTAGACACCAGCAAGATGGTCGACTG
CTCCGAGCTCATTCCTGTTCCTCCTGAGCTGAAGATCACCGCCGCGCATTTCCCTGCTGGCAA
GACCAACGCTGACGTCGAGCAAGCTTGTGCTTCGACCCCCTTCCCCACTCTGTCCACTGACCC
CGGCCCGGCTACTTCTGTCGCTCCTGTCCCTCCGTCCTAA

[0033] The mature manganese peroxidase MnP-5 protein has a theoretical molecular weight of 36.5kDa and is a novel manganese peroxidase.

SEQ ID NO.28(MnP-6)：

ATGGCCTTCAAACAACTCGTCGCTGCACTTACAGTCGCGCTGTCACTCGGTGTTGCACA
AGGTGCTATCACCAGACGTGTTGCGTGCCCCGACGGCGTGAACACGGCCACCAACGCGGCCT
GTTGTTCTTTGTTCGCCATTCGTGATGATATCCAACAAAACCTCTTCGACGGTGGTGAATGTGG
GGAGGAGGTTCACGAGTCTTTCCGTCTGACCTTCCATGATGCCATCGGCATTGGCTCAAACGG
TGGCGGAGGTGCTGACGGCTCCATTGCTGTTTTCGAGGACATTGAGACCGCTTTCCACGCCA
ACAACGGTGTCGACGAAATCATCGACGAGCAGAAGCCGTTCCTCGCCAGACACAACATCACC
CCCGGTGATTTCATTCAATTCGCTGGTGCTGTCGGTGTCTCCAACTGTCCCGGTGCTCCTCGTC
TCGATTTCTTCCTCGGCCGACCAAACCCGGTCGCTCCTGCTCCTGACAAGACCGTTCCTGAGC
CTTTCGATACTGTTGACAGCATTCTGGCTCGCTTCGCGGATGCTGGTGGATTCAGCCCGGCTG
AGGTTGTCGCTCTTCTTGGATCCCACACGATCGCTGCAGCCGATCATGTTGACCCGACCATCCC
TGGTACACCTTTCGACTCTACTCCTGAGGTGTTCGACACCCAGGTGTTCCTTGAAGTCCAGCT
TCGTGGAACGCTCTTCCCCGGAACTGGTGGAAACCAGGGTGAAGTTGAGTCTCCTCTTCGTG
GTGAAATCCGTCTTCAGTCTGACCATGACCTCGCCCGTGACTCGAGGACGGCTTGCGAATGG
CAGTCGTTCGTGAACAATCAAGTCAAGCTTCAGACTGCCTTCAAGGCCGCTTTCAAGAAGCTC
GCTGTACTCGGCCACGATGTCAACAACATGGTTGACTGCTCCGAAGTCATCCCCGAGCCCCCG
AACGTCAAGATCAAGGCCGCGACCTTCCCCGCTGGCCAGACCAACGCCGATGTTGAGCAGG

CTTGCGCCTCCACTCCCTTCCCCACTCTTGCTACTGACCCCGGCCCGGCTACCTCCGTTGCCCC
TGTTCCCCCGTCTTAA

According to an embodiment, gene encoding manganese peroxidase MnP-6 was cloned by PCR, and the analysis of its DNA sequence showed genomic sequence had full length of 1580bp, including encoding sequence of 1080bp, and

an oligonucleotide sequence as below, encoding the signal peptide.

SEQ ID NO.29

ATGGCCTTCAAACAACTCGTCGCTGCACTTACAGTCGCGCTGTCACTCGGTGTTGCACA AGGT

[0034] The cDNA sequence of the mature manganese peroxidase MnP-6 has a nucleotide sequence set in forth in SEQ ID NO.30

SEQ ID NO.30：

Gctatcaccagacgtgttgcgtgccccgacggcgtgaacacggccaccaacgcggcctgttgttctttgttcgccattcgtg atgatatccaacaaaacctcttcgacggtggtgaatgtggggaggaggttcacgagtctttccgtctgaccttccatgatgc catcggcattggctcaaacggtggcggaggtgctgacggctccattgctgttttcgaggacattgagaccgctttccacgcc aacaacggtgtcgacgaaatcatcgacgagcagaagccgttcctcgccagacacaacatcacccccggtgatttcattca attcgctggtgctgtcggtgtctccaactgtcccggtgctcctcgtctcgatttcttcctcggccgaccaaacccggtcgctcc tgctcctgacaagaccgttcctgagcctttcgatactgttgacagcattctggctcgcttcgcggatgctggtggattcagcc cggctgaggttgtcgctcttcttggatcccacacgatcgctgcagccgatcatgttgacccgaccatccctggtacacctttc gactctactcctgaggtgttcgacacccaggtgttccttgaagtccagcttcgtggaacgctcttccccggaactggtggaa accagggtgaagttgagtctcctcttcgtggtgaaatccgtcttcagtctgaccatgacctcgcccgtgactcgaggacggc ttgcgaatggcagtcgttcgtgaacaatcaagtcaagcttcagactgccttcaaggccgctttcaagaagctcgctgtactc ggccacgatgtcaacaacatggttgactgctccgaagtcatccccgagcccccgaacgtcaagatcaaggccgcgacctt ccccgctggccagaccaacgccgatgttgagcaggcttgcgcctccactcccttccccactcttgctactgaccccggcccg gctacctccgttgcccctgttccccgtcttaa

[0035] The mature manganese peroxidase MnP-6 protein has a theoretical molecular weight of 35.6kDa and is a novel manganese peroxidase.

[0036] In another aspect, the present invention provides a derived the manganese peroxidases by substitution, deletion and/or insertion of one or more amino acid residues to the amino acid sequence as shown in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.10 or SEQ ID NO.13, and maintaining the ability of detoxifying mycotoxin.

[0037] In a preferred embodiment, a manganese peroxidase is such an active protein having at least about 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the full amino acid sequence as shown in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.10 or SEQ ID NO.13.

[0038] In another aspect, the present invention provides a derived the manganese peroxidases by substitution, deletion and/or insertion of one or more amino acid residues to the amino acid sequence as shown in SEQ ID NO.3, SEQ ID NO.6, SEQ ID NO.9, SEQ ID NO.12 or SEQ ID NO.15, and maintaining the ability of detoxifying mycotoxin.

[0039] In a preferred embodiment, a manganese peroxidase is such an active protein having at least about 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the full amino acid sequence as shown in SEQ ID NO.3, SEQ ID NO.6, SEQ ID NO.9, SEQ ID NO.12 or SEQ ID NO.15, and maintaining the ability of detoxifying mycotoxin.

[0040] Yet another aspect of the invention is to provide genes encoding the above manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 or MnP-6, selected from

(a) DNA comprising a nucleotide sequence set in forth in SEQ ID NO.16, SEQ ID NO.18, SEQ ID NO.19, SEQ ID NO.21, SEQ ID NO.22, SEQ ID NO.24, SEQ ID NO.25, SEQ ID NO.27, SEQ ID NO.28 or SEQ ID NO.30; or
(b) DNA having a nucleotide sequence at least about 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%

or 99% homology to that shown in SEQ ID NO.16, SEQ ID NO.18, SEQ ID NO.19, SEQ ID NO.21, SEQ ID NO.22, SEQ ID NO.24, SEQ ID NO.25, SEQ ID NO.27, SEQ ID NO.28 or SEQ ID NO.30, and encoding the proteins with the same function as that encoded by the above DNA comprising a nucleotide sequence set in forth in SEQ ID NO.16, SEQ ID NO.18, SEQ ID NO.19, SEQ ID NO.21, SEQ ID NO.22, SEQ ID NO.24, SEQ ID NO.25, SEQ ID NO.27, SEQ ID NO.28 or SEQ ID NO.30, wherein, the nucleotide sequence homologous to the above sequence may be codon-optimized sequences, sequences added with cleavage sites, or other known modified sequences in the art.

[0041] In another aspect, the present invention provides the recombinant vector containing the genes encoding manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6. According to an embodiment of the present invention, said recombinant vectors containing the genes encoding manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6 are the vector pET28a-MnP-1, the vector pET28a-MnP-2, the vector pET28a-MnP-4, the vector pET28a-MnP-5 and the vector pET28a-MnP-6. In a preferred embodiment of the present invention, the genes encoding manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6 are inserted between the sites of BamHI and Not I, BamHI and XhoI, BamHI and XhoI, EcoRI and Xho, and EcoRI and NotI of the vector pPIC9, respectively, to under the control and regulation of the promoter T7 to obtain the recombinant expression vectors pET28a-MnP-1, pET28a-MnP-2, pET28a-MnP-4, pET28a-MnP-5 and pET28a-MnP-6.

[0042] The present invention provides recombinant strains comprising the above the genes encoding manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6. According to the embodiment of the present invention, said recombinant strains are the Escherichiacoli strain BL21 (DE3)/MnP-1, BL21(DE3)/MnP-2, BL21(DE3)/MnP-4, BL21(DE3)/MnP-5 and BL21(DE3)/MnP-6.

[0043] Accordingly, the invention further provides method for producing manganese peroxidase MnP-1, MnP-2, MnP-4, MnP-5 or MnP-6. In one embodiment, the method comprises steps of transforming the host cell with the above recombinant vectors to obtain the recombinant strains, culturing the recombinant strains to induce expression of recombinant manganese peroxidase, and refolding and isolating the protein.

[0044] In a preferred embodiment of the present invention, the method includes the step of transforming the Ecoli cells with the recombinant Ecoli expression plasmids to obtain the recombinant strains.

[0045] In a preferred embodiment of the present invention, the method of the present invention incudes step of transforming the Ecoli cells BL21(DE3) with the recombinant Ecoli expression plasmids to obtain recombinant Ecoli BL21(DE3)/MnP-1, BL21(DE3)/MnP-2, BL21(DE3)/MnP-4, BL21(DE3)/MnP-5 and BL21(DE3)/MnP-6.

[0046] In another aspect, the present invention provides the application of the above manganese peroxidase MnP-1, MnP-2, MnP-4, MnP-5 or MnP-6 to detoxify mycotoxin.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0047]

FIG.1 shows degradation rates of aflatoxin by recombinant manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6.

FIG.2 shows degradation rates of zearalenone by recombinant manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6.

FIG.3 shows degradation rates of vomitoxin by recombinant manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6.

FIG.4 shows HPLC analysis results of the degradation of aflatoxin by recombinant manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6

FIG.5 shows HPLC analysis results of the degradation of zearalenone by recombinant manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6.

FIG.6 shows HPLC analysis results of the degradation of vomitoxin by recombinant manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6.

EMBODIMENT

[0048] The present invention is further illustrated with reference to the following Examples and the appended drawings,

which should by no means be construed as limitations of the present invention.

Test materials and reagents

[0049]

1. Strains and vectors: Irpex lacteus from which the five genes encoding manganese peroxidases MnP-1, MnP-2, MnP-4, MnP-5 and MnP-6 were cloned respectively, the Ecoli expression vectors pET-28a(+) and strain BL21(DE3) purchased from Invitrogen.

2. Enzymes and other biochemical reagents: restriction endonucleases (Fermentas), ligase (Invitrogen), aflatoxin (Aladdin), zearalenone and vomitoxin (Sigma-Aldrich), the other reagents available purchased.

3. Medium:

(1) Irpex lacteus producing enzyme medium: 1% of lignocellulose, 0.2g/L of ammonium tartrate, 2g/L of KH_2PO_4 0.71g/L of $MgSO_4 \cdot 7H_2O$, 0.1g/L of $CaCl_2$, 70mL of macroelements concentrate.

(2) Microelement solution: 1g/L of NaCl, 0.184g/L of $CoCl_2 \cdot 6H_2O$, 0.1g/L of $FeSO_4 \cdot 7H_2O$, 0.1g/L of $ZnSO_4 \cdot 7H_2O$, 0.1g/L of $CuSO_4$, 0.01g/L of H_3BO_3, 0.01g/L of $Na_2MoO_4 \cdot 2H_2O$, 0.01g/L of $KAl(SO_4)_2 \cdot 12H_2O$, 1.5g/L of nitrilotriacetic acid.

(3)E.coli. LB medium: 1% of peptone, 0.5% of yeast extract, and 1% of NaCl, natural pH.

[0050] Suitable biology laboratory methods not particularly mentioned in the examples as below can be found in Sambrook, et al. (Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other kit laboratory manuals.

Example 1 cloning gene encoding manganese peroxidase MnP-1, MnP-2, MnP-4, MnP-5and MnP-6 from Irpex lactus

Isolating the total RNA of Irpex lactus

[0051] First, bacteria cells cultured in enzyme-producing medium for 3 days were collected on the filter paper and pressed dry, followed by adding liquid nitrogen to a high-temperature sterilized mortar and quickly ground the bacteria into powder. Then, the grounded powder was transferred to a centrifuge tube with 800μL of Trizol, blended well and left in the room temperature for 5 min. 200L of chloroform was added, shaken violently for 15s, placed at room temperature for 3 min, and centrifuged at 4°C at 12,000 RPM for 15 min. The supernatant was obtained, and isopropanol of the equal volume was added to be mixed well, placed at room temperature for 10 min and centrifuged at 4°C at 12,000 RPM for 10 min. The supernatant was removed and the precipitation was washed twice with 70% of ethanol followed by drying in the air for 5 min, and an appropriate amount of DNase/ Rnase-free deionized water was added to dissolve RNA.

[0052] The specific primers for manganese peroxidase gene were synthesized as below.

MnP-1 :

P1:5'-CGCGGATCCGCACCCTCTTCTAGAGTGACATGCAGT-3';
P2:5'-TAAAGCGGCCGCTTACACAGGAACGATGGAGGTGGCG-3'.

MnP-2 :

P3:5'-CGCGGATCCGCAATCACCAAGCGTGTTGCTTGTCCT-3';
P4:5'-CCGCTCGAGTTACGAGGGAGGGACAGGGGCGACAGA-3'.

MnP-4 :

P5:5'-CGCGGATCCGCTCCCCAAGACGTTACTGCCGC-3';
P6:5'-CCGCTCGAGTTACGACGGAGGTACTGGAGGAATCG-3'.

MnP-5 :

P7:5'-CGGAATTCGCCGTCGTCAGGCGTGTCACTTG-3';
P8:5'-CCGCTCGAGTTAGGACGGAGGGACAGGAGCGAC-3'.

MnP-6 :

P9:5'-CGGAATTCGCTATCACCAGACGTGTTGCGTGC-3';
P10:5'-ATTTGCGGCCGCTTAAGACGGGGGAACAGGGGCAAC-3'.

[0053] PCR amplification was performed with cDNA obtained by RT-PCR using the total RNA of Irpex lacteu. PCR reaction parameters were denaturation at 95°C for 5min, followed by 35 cycles of denaturing at 94°C for 30sec, annealing at 55°C for 30sec, and extending at 72°C for 1min, and being kept at 72°C for 10min. After electrophoresis on 1% of agarose gel, the target fragment was cut, recovered and connected with vector pEASY-T3 for sequencing.

Example 2 Preparing the recombinant manganese peroxidases

[0054] The expression vectors pET28a-MnP-1, pET28a-MnP-2, pET28a-MnP-4, pET28a-MnP-5 and pET28a-MnP-6 were constructed by connecting the gene encoding the mature manganese peroxidases NMP-1,NMP-2,NMP-4,NMP-5 and MnP-6 with the expression vector ET-28a(+), both of which were digested with restriction enzymes, and were transformed to Ecoli strain BL21(DE3) to obtain the recombinant strains BL21(DE3)/MnP-1, BL21(DE3)/MnP-2, BL21(DE3)/MnP-4, BL21(DE3)/MnP-5 and BL21(DE3)/MnP-6.

[0055] The strain D3 containing the recombinant plasmid was planted into 40 mL of LB culture medium for culturing at 37°C for 12h, followed by being planted into 300 mL of LB culture medium at a ratio of 2% for culturing for 4h at 37°C with 250rpm, with addition of inducer IPTG in the final concentration of 1mM to induce for 4h when reaching to 0.8 of OD_{600}, and collecting bacteria by centrifuging. The bacteria cells were lysed by Lysozyme using 8M of urea to dissolve inclusion body protein, and the refolding system prepared with 50mM of Tris-HCl buffer with 9.5 of pH , 0.5m M of urea, 0.5 mM of GSSG, 0.1 mM of DTT, 10 μ M of hemin, 5mM of $CaCl_2$, and 0.1mg/mL of protein solution, for renaturing for 10h at 15°C. After the renaturated manganese peroxidase was purified, the content of protein reached to more than 95% of the total protein.

Example 3 Degradation of aflatoxin by the recombinant manganese peroxidase

[0056] Aflatoxin was dissolved into 50mg/L of mother liquor of dimethyl sulfoxide to react for 10h at 30°C in the reaction system of 70 μl of malonic acid buffer (0.2m, pH 5.0), 20 μl of aflatoxin solution, 5 μl of manganese sulfate (40mM), 100 μl of manganese peroxidase (1000U/L), 5 μl of hydrogen peroxide (4mM), taking the system without manganese peroxidase as control, wherein each manganese peroxidase was set three repeats. The reaction was terminated by adding DMSO in three times of volume, to measure the degradation rate of aflatoxin in wavelength of 365nm by high performance liquid chromatography (HPLC) using Nexera UHPLC system of which the chromatographic column is Zorbax sb-c18 (4.6X 250,5um), the mobile phase A was 0.06% of TFA water, and the mobile phase B was 0.05% TFA acetonitrile, and eluted with gradient content of 30% of solution B for 4 min, 30%-100% of solution B for 15 min, and 100% of solution B for 5 min.

Example 4 Degradation of zearalenone by the recombinant manganese peroxidase

[0057] Zearalenone was dissolved into 50mg/L of mother liquor of dimethyl sulfoxide to react for 10h at 30°C in the reaction system of 70 μl of malonic acid buffer (0.2m, pH 5.0), 20 μl of aflatoxin solution, 5 μl of manganese sulfate (40mM), 100 μl of manganese peroxidase (1000U/L), 5 μl of hydrogen peroxide (4mM), taking the system without manganese peroxidase as control, wherein each manganese peroxidase was set three repeats. The reaction was terminated by adding DMSO in three times of volume, to measure the degradation rate of zearalenone in wavelength of 365nm by high performance liquid chromatography (HPLC) using Nexera UHPLC system of which the chromatographic column is Zorbax sb-c18 (4.6X 250,5um), the mobile phase A was 0.06% of TFA water, and the mobile phase B was 0.05% TFA acetonitrile, and eluted with gradient content of 30% of solution B for 4 min, 30%-100% of solution B for 15 min, and 100% of solution B for 5 min.

Example5 Degradation of v by the recombinant manganese peroxidase

[0058] Vomitoxin was dissolved into 50mg/L of mother liquor of dimethyl sulfoxide to react for 10h at 30°C in the

reaction system of 70 μl of malonic acid buffer (0.2m, pH 5.0), 20 μl of aflatoxin solution, 5 μl of manganese sulfate (40mM), 100 μl of manganese peroxidase (1000U/L), 5 μl of hydrogen peroxide (4mM), taking the system without manganese peroxidase as control, wherein each manganese peroxidase was set three repeats. The reaction was terminated by adding DMSO in three times of volume, to measure the degradation rate of vomitoxin in wavelength of 365nm by high performance liquid chromatography (HPLC) using Nexera UHPLC system of which the chromatographic column is Zorbax sb-c18 (4.6X 250,5um), the mobile phase A was 0.06% of TFA water, and the mobile phase B was 0.05% TFA acetonitrile, and eluted with gradient content of 30% of solution B for 4 min, 30%-100% of solution B for 15 min, and 100% of solution B for 5 min.

Claims

1. Application of manganese peroxidase to detoxification of mycotoxin.

2. The application according to claim 1, being **characterized in that** said manganese peroxidase is selected from

   a) a polypeptide having the amino acid sequence as set in forth in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.10, or SEQ ID NO.13;
   b) a polypeptide comprising the amino acid sequence obtained by substituting, deleting, and or inserting one or more amino acid residues in the amino acid sequence depicted in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.10 or SEQ ID NO.13, and maintaining the ability of detoxifying mycotoxin; or
   c) a polypeptide having at least 70% identity to the amino acid sequence depicted in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.10or SEQ ID NO.13, and maintaining the ability of detoxifying mycotoxin.

3. The application according to claim 1 or 2, being **characterized in that** said manganese peroxidase is a mature protein selected from

   a) a polypeptide having the amino acid sequence as set in forth in SEQ ID NO.3, SEQ ID NO.6, SEQ ID NO.9, SEQ ID NO.12, or SEQ ID NO.15;
   b) a polypeptide comprising the amino acid sequence obtained by substituting, deleting, and or inserting one or more amino acid residues in the amino acid sequence depicted in EQ ID NO. 3, SEQ ID NO. 6, SEQ ID NO.9, SEQ ID NO.12, or SEQ ID NO.15, and maintaining the ability of detoxifying mycotoxin; or
   c) a polypeptide having at least 70% identity to the amino acid sequence defined by "a)", and maintaining the ability of detoxifying mycotoxin.

4. Manganese peroxidase being **characterized in that** said manganese peroxidases are selected from

   a) a polypeptide having the amino acid sequence as set in forth in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.10 or SEQ ID NO.13, and the ability of detoxifying mycotoxin;
   b) a polypeptide comprising the amino acid sequence obtained by substituting, deleting, and or inserting one or more amino acid residues in the amino acid sequence depicted in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.10 or SEQ ID NO.13, and maintaining the ability of detoxifying mycotoxin; or
   c) a polypeptide having at least 70% identity to the amino acid sequence defined by "a)", and maintaining the ability of detoxifying mycotoxin.

5. The manganese peroxidase according to claim 5 being **characterized in that** said manganese peroxidase is a mature protein selected from

   a) a polypeptide having the amino acid sequence as set in forth in SEQ ID NO.3, SEQ ID NO.6, SEQ ID NO.9, SEQ ID NO.12, or SEQ ID NO.15, and the ability of detoxifying mycotoxin;
   b) a polypeptide comprising the amino acid sequence obtained by substituting, deleting, and or inserting one or more amino acid residues in the amino acid sequence depicted in EQ ID NO. 3, SEQ ID NO. 6, SEQ ID NO.9, SEQ ID NO.12, or SEQ ID NO.15, and maintaining the ability of detoxifying mycotoxin; or
   c) a polypeptide having at least 70% identity to the amino acid sequence defined by "a)", and maintaining the ability of detoxifying mycotoxin.

6. Gene encoding the manganese peroxidase of claim 4 or 5.

7. The gene according to claim 6, being characterized of

a) having the nucleotide sequence as set in forth in SEQ ID NO.16, SEQ ID NO. 19, SEQ ID NO. 22, SEQ ID NO. 25 or SEQ ID NO. 28;
b) having the nucleotide sequence as set in forth in SEQ ID NO.18 , SEQ ID NO. 21, SEQ ID NO.24, SEQ ID NO. 27 or SEQ ID NO.30; or
c) having the nucleotide sequence at least 70% identity to the nucleotide sequence defined by "a)" or "b)", and encoding the protein having the same function as that encoded by gene defined in "a)" or "b)".

8. Recombinant vector containing the gene of claim 6 or 7.

9. Recombinant strain containing the gene of claim 6 or 7, or the recombinant vector of claim 8.

10. A method for preparing the manganese peroxidase of claim 4 or 5, being **characterized in that** said method includes the steps of

1) transforming a host cell with the recombinant vector of claim 8 to obtain a recombinant strain;
2) culturing the said recombinant strain to induce express recombinant manganese peroxidase; and
3) purifying the said manganese peroxidase.

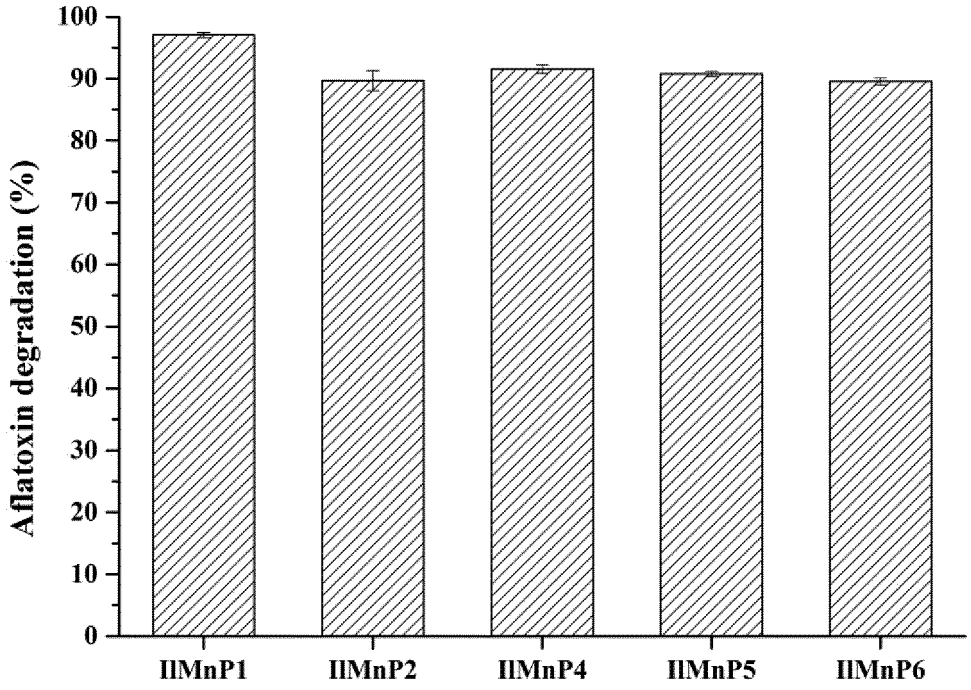
Aflatoxin degradation (%)
100
90
80
70
60
50
40
30
20
10
0
IlMnP1
IlMnP2
IlMnP4
IlMnP5
IlMnP6

FIG 1

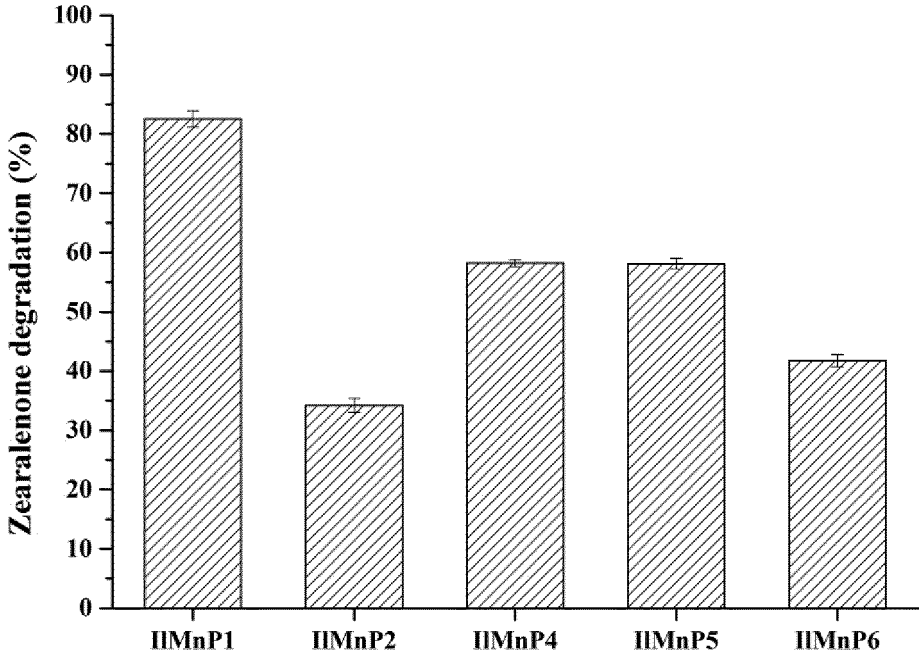
Zearalenone degradation (%)
100
90
80
70
60
50
40
30
20
10
0
IlMnP1
IlMnP2
IlMnP4
IlMnP5
IlMnP6

FIG2

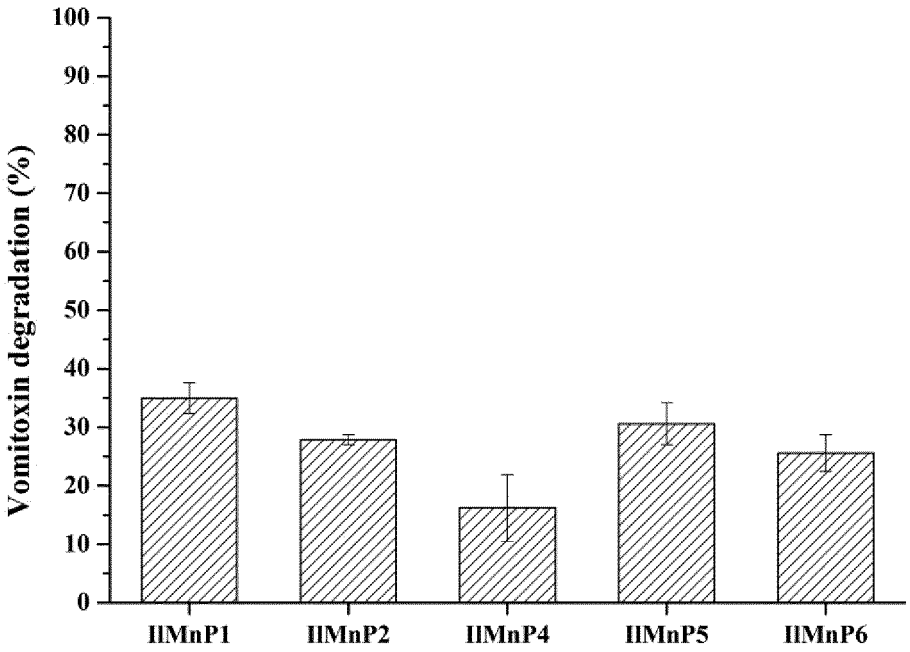
Vomitoxin degradation (%)
100
90
80
70
60
50
40
30
20
10
0
IlMnP1
IlMnP2
IlMnP4
IlMnP5
IlMnP6

FIG 3

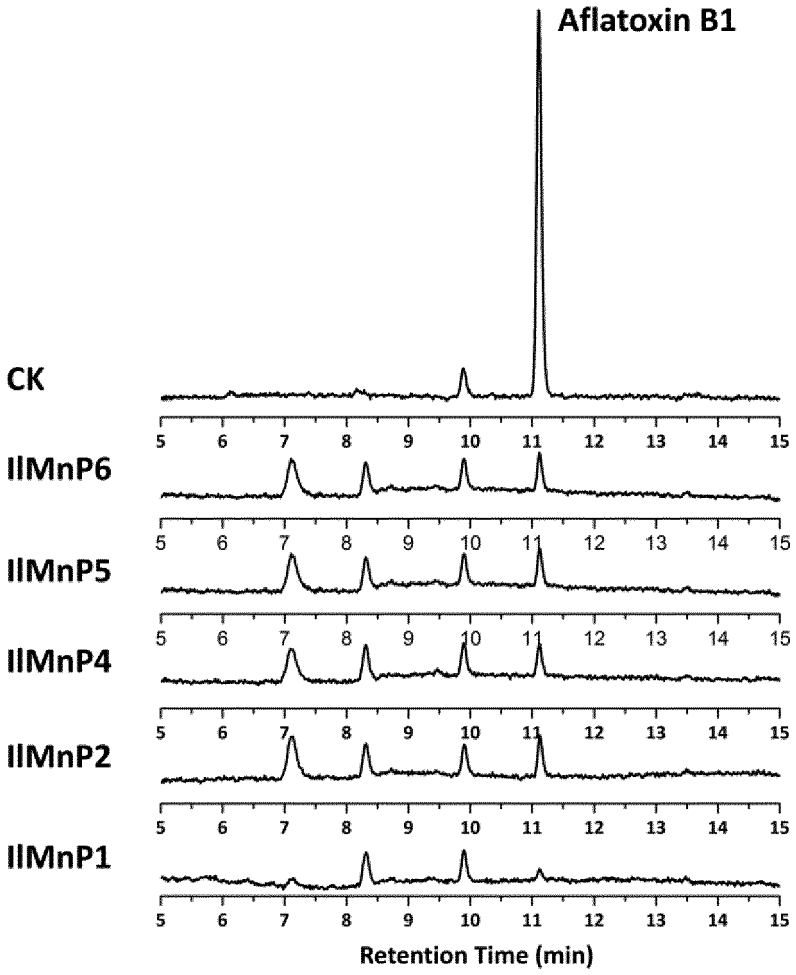
Aflatoxin B1
CK
IlMnP6
IlMnP5
IlMnP4
IlMnP2
IlMnP1
5 6 7 8 9 10 11 12 13 14 15
Retention Time (min)

FIG4

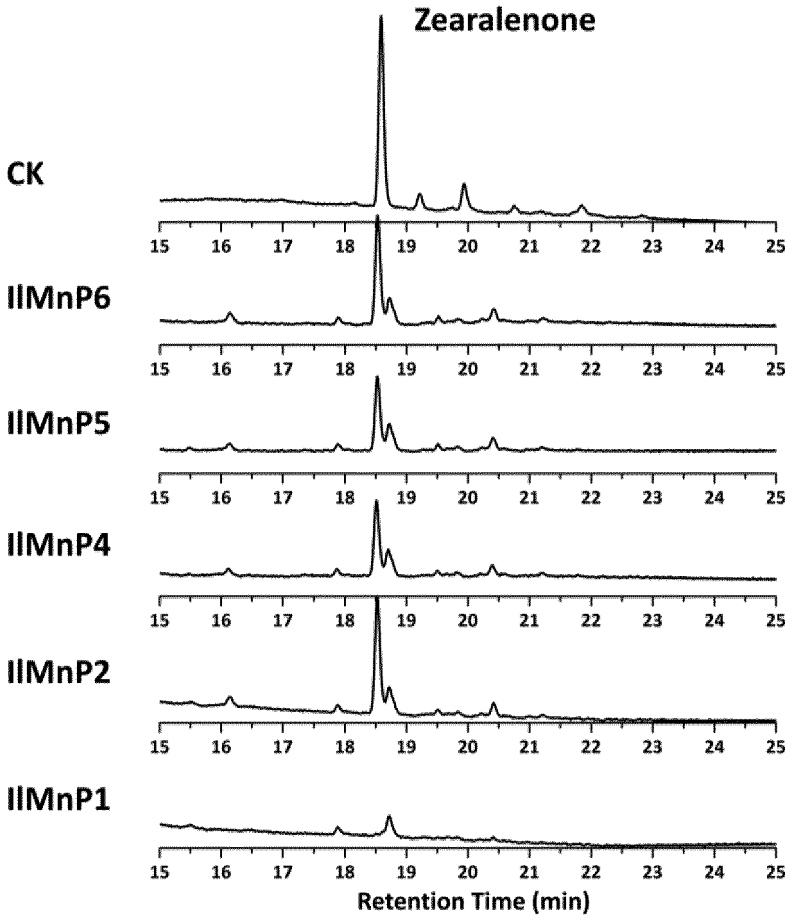
Zearalenone
CK
IlMnP6
IlMnP5
IlMnP4
IlMnP2
IlMnP1
15 16 17 18 19 20 21 22 23 24 25
Retention Time (min)

FIG 5

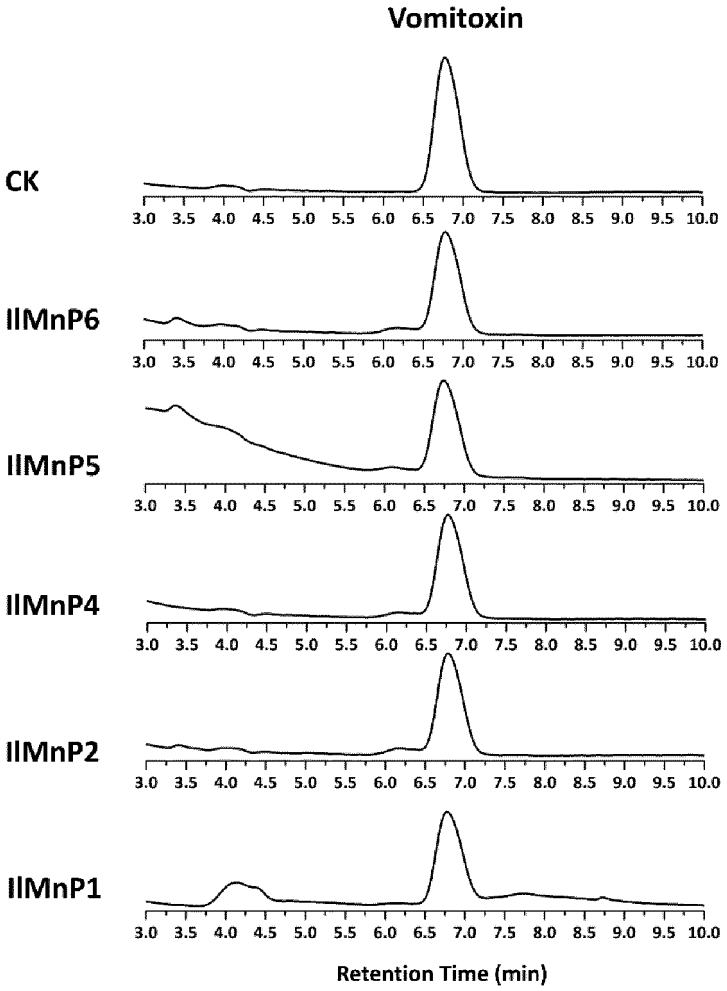
Vomitoxin
CK
IlMnP6
IlMnP5
IlMnP4
IlMnP2
IlMnP1
3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0
Retention Time (min)

FIG 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/086534

A. CLASSIFICATION OF SUBJECT MATTER

C12N 9/08 (2006.01) i; C12N 15/53 (2006.01) i; C12N 15/70 (2006.01) i
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI, SIPOABS, CNABS, CNKI, ISI Web of knowledge, NCBI, Google Scholar, GenBank, NATIONAL BIO-SEQUENCE DATABASE OF CHINESE PATENT: 锰过氧化物酶, 乳白耙菌, 霉菌, 毒素, 黄曲霉, manganese peroxidase, Irpex lactrus, mould, toxin, aspergillus, aflatoxin, search for sequences 1, 3, 16 and 18

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	CN 107012131 A (FEED RESEARCH INSTITUTE, CHINESE ACADEMY OF AGRICULTURAL SCIENCES), 04 August 2017 (04.08.2017), claims	1-10
X	WANG, Jianqiao, et al. "Detoxification of Aflatoxin B1 by Manganese Peroxidase from the White-rot Fungus Phanerochaete Sordida YK-624", FEMS Microbiology Letters, 314(2), 31 January 2011 (31.01.2011), abstract	1
X	YEHIA, R.S., "Aflatoxin Detoxification by Manganese Peroxidase Purified from Pleurotus Ostreatus", Brazilian Journal of Microbiology, 45(1), 01 May 2014 (01.05.2014), abstract	1
A	WANG, Jianqiao, et al. "Detoxification of Aflatoxin B1 by Manganese Peroxidase from the White-rot Fungus Phanerochaete Sordida YK-624", FEMS Microbiology Letters, 314(2), 31 January 2011 (31.01.2011), entire document	2-10

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 November 2017	26 February 2018
Name and mailing address of the ISA State Intellectual Property Office of the P. R. China No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088, China Facsimile No. (86-10) 62019451	Authorized officer LI, Lan Telephone No. (86-10) 62411619

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/086534

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YEHIA, R.S., "Aflatoxin Detoxification by Manganese Peroxidase Purified from Pleurotus Ostreatus", Brazilian Journal of Microbiology, 45(1), 01 May 2014 (01.05.2014), entire document	2-10
A	CN 104232555 A (SHANGHAI JIAOTONG UNIVERSITY), 24 December 2014 (24.12.2014), entire document	1-10
A	YU, Cun and CHI, Yujie, "AFK91528.1 Manganese Peroxidase 1 (Cerrena Unicolor)", Genbank, 09 June 2012 (09.06.2012), entire document	1-10

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/086534

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☒ on paper

   ☐ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Form PCT/ISA/210 (continuation of first sheet (1)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/086534

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

[1] 1) claims 1-10 (part): manganese peroxidases of SEQ ID NO: 1 and SEQ ID NO: 3 and an application thereof, coding genes SEQ ID NO: 16 and SEQ ID NO: 18 of the manganese peroxidases, and corresponding vectors, recombinant strains and a preparation method;

[2] 2) claims 1-10 (part): manganese peroxidases of SEQ ID NO: 4 and SEQ ID NO: 6 and an application thereof, coding genes SEQ ID NO: 19 and SEQ ID NO: 21 of the manganese peroxidases, and corresponding vectors, recombinant strains and a preparation method;

[3] 3) claims 1-10 (part): manganese peroxidases of SEQ ID NO: 7 and SEQ ID NO: 9 and an application thereof, coding genes SEQ ID NO: 22 and SEQ ID NO: 24 of the manganese peroxidases, and corresponding vectors, recombinant strains and a preparation method;

[4] 4) claims 1-10 (part): manganese peroxidases of SEQ ID NO: 10 and SEQ ID NO: 12 and an application thereof, coding genes SEQ ID NO: 25 and SEQ ID NO: 27 of the manganese peroxidases, and corresponding vectors, recombinant strains and a preparation method; and

[5] claims 1-10 (part): manganese peroxidases of SEQ ID NO: 13 and SEQ ID NO: 15 and an application thereof, coding genes SEQ ID NO: 28 and SEQ ID NO: 30 of the manganese peroxidases, and corresponding vectors, recombinant strains and a preparation method.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: claims 1-10 (part): manganese peroxidases of SEQ ID NO: 1 and SEQ ID NO: 3 and an application thereof, coding genes SEQ ID NO: 16 and SEQ ID NO: 18 of the manganese peroxidases, and corresponding vectors, recombinant strains and a preparation method

Remark on protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2017/086534

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN 107012131 A	04 August 2017	None	
CN 104232555 A	24 December 2014	None	

Form PCT/ISA/210 (patent family annex) (July 2009)

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0050]**